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TECHNICAL NOTE

Accuracy of measurements of HbF with OSM3 in neonates and infants

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Accuracy of measurements of HbF with OSM3 in neonates and infants. A. Denjean, F. Bridey, J.P. Praud, J.F. Magny, M. Dehan, Cl. Gaultier.

ABSTRACT: The accuracy of the Radiometer OSM3 oxymeter for measurement of fetal haemoglobin (HbF) in infants was investigated, and compared to one of the standard reference methods using alkali electrophoresis of haemoglobin. Blood samples of 37 infants with different gestational (27–41 weeks) and postnatal (1–198 days) ages were analysed. The two methods gave very close results but a significant mean difference (range -4.5–16.5%). However, agreement between the two methods was judged clinically acceptable (95% limits of agreement -7.5–15.5%). A rapid determination of HbF percentage, using OSM3, is an important determinant for correct assessment of oxygen saturation in newborn infants in intensive care units.

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Percentage of fetal haemoglobin (HbF) is an important determinant for haemoglobin oxygen saturation (Sao_2). High HbF percentages can induce errors in determining Sao_2 as current co-oxymeters can mistake HbF for carboxyhaemoglobin [1].

One of the standard reference methods for accurate measurement of HbF requires alkaline electrophoresis of haemoglobin [2]. This method is time-consuming and is, therefore, not suitable for routine use in neonate management.

Recently, FOGH-ANDERSEN *et al.* [3] reported accurate results of haemoglobin pigments in healthy newborns with 80±5% HbF, using a direct spectrophotometric determination (Radiometer OSM3, Copenhagen, DK), with an adapted matrix of absorption coefficients for neonatal blood. From their results the authors suggested that this apparatus may be used to directly estimate the ratio of fetal to total haemoglobin in infants.

The HbF % in fetal blood largely depends on maturity and postnatal age. The aim of this study was, therefore, to determine the accuracy of OSM3 measurements of HbF %, in infants having different maturity and postnatal age, with expected levels of HbF between 0–100%.

Subjects and methods

The study involved 37 neonates and infants being treated for acute respiratory disorders. The mean gestational age was 33 weeks (range 27–41 weeks) and the mean postnatal age was 34 days (range 1–198 days). Twenty nine of the patients required mechanical ventilation and 32 received supplemental oxygen. As part of the clinical management, blood samples (1.5–2 ml) were drawn for blood gas analysis. Sampling was done with a heparinized syringe either through an indwelling umbilical arterial catheter or by radial or tibial puncture. After blood gas analysis, the remainder of the sample was separated into two aliquots: one for HbF determination with OSM3 haemoxymeter, the second for HbF determination using the standard reference method.

OSM3 measurement of HbF %

Each HbF measurement was repeated using the following protocol: 200–400 µl blood were fully oxygenated for 60 s, using a vortex. One hundred µl of oxygenated blood were then introduced into the OSM3 haemoxymeter to obtain the HbF % measurement. Briefly, the principle is as follows: fully oxygenated adult blood (0% HbF) gives a result of 100% Hb oxygen saturation, while fully oxygenated newborn blood (with HbF = 80%) gives a result of 104%. From this, the percentage of HbF can be automatically determined.

Measurement of HbF by reference method

HbF was determined by the reference method (HbF_{STD}) of electrophoresis on alkali gel (Ciba-Corning, USA) [2]. In addition, isoelectric focusing in polyacrylamide gel at pH 5.5–8.5 (LKB, Brommo, Sweden) was used to confirm the result and rule out any other

Hb abnormality [4]. In our laboratory, repeatability of measurements of HbF by electrophoresis on alkali gel has been estimated on ten consecutive measurements in two different samples containing 53.6 and 79.6% HbF. Coefficients of variation for the two sets of measurements were 3.29% and 3.45%, respectively.

Statistical analysis

Data were analysed using Student's paired t-test when appropriate. The mean difference (bias) between HbF % measured by OSM3 (HbF_{OSM3}) and reference method (HbF_{STD}) was calculated, as was the standard deviation of the difference (precision). Agreement between the two methods was assessed using a plot of the differences observed, against average for HbF % data by the two methods. The limits of agreement were then calculated as the 95% confidence intervals for the bias [5, 6]. Data are expressed as mean±SD.

Measurement of HbF by reference method

The mean of HbF_{STD} was 52.1±32.9%, with a range of 0–98%. The isoelectric focusing technique confirmed this result and did not show any abnormal haemoglobin in the samples.

Comparison between HbF_{OSM3} and HbF_{STD}

Although measurements of HbF_{OSM3} and HbF_{STD} were very close, the mean of HbF_{OSM3} and HbF_{STD} were significantly different with a mean difference of 4.1±5.8% (range -4.5–16.5, t=4.3, p<0.001). The confidence interval for the bias was 2.1–5.9%. Figure 1 shows the differences between HbF_{OSM3} and HbF_{STD} against the average HbF % using the two methods. These differences were not related to the average. The 95% limits of agreement were calculated as mean difference ±2 SD = -7.5–15.5%, *i.e.* HbF_{OSM3} may be 7.5% below or 15.5% above HbF_{STD}.

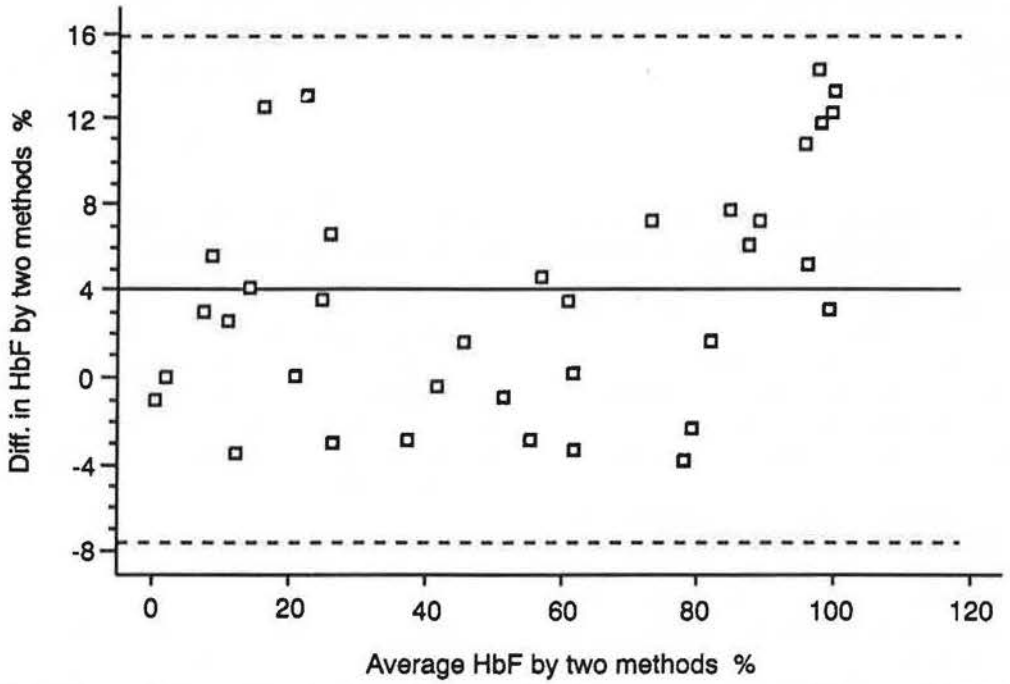

Fig. 1. – Paired difference (HbF_{OSM3} - HbF_{STD}) against paired mean. Dotted lines represent ±SD around the mean (solid line). HbF: fetal haemoglobin; HbF_{STD}: HbF measured by the reference method of electrophoresis on alkali gel; HbF_{OSM3}: HbF measured by OSM3 oxymeter.

Results

Measurement of HbF by OSM3

Duplicate measurements of HbF_{OSM3} showed no significant difference (mean difference -1±3%, t_{36}=-2, NS), and the coefficient of repeatability was 6%. Based on this, the mean of each duplicate measurement was used for statistical analysis. The mean value for the population was 56.2±35.2% with a range of 0–107%. Five of the 37 measurements showed results exceeding 100% HbF.

Discussion

In newborns with a range of HbF from 0–98%, we found that the OSM3 is able to measure HbF with a bias of 4.1% and a precision of 5.8%.

In 37 duplicate measurements with the OSM3, the precision was 3%, *i.e.* twice the precision of the reference methods in the study of FOGH-ANDERSEN *et al.* [3].

The repeatability of OSM3 measurements, expressed by the variation coefficient, was 6%, *i.e.* about twice the variation coefficient of HbF established in our

laboratory with the reference method. This discrepancy is probably due to the difference in the number of repetitions (two for OSM3, ten for HbF by reference method).

In the study of FOGH-ANDERSEN *et al.* [3] based on newborns with 80±5% HbF, the precision was 2.1% when HbF measurements by the reference method and OSM3 were compared. In our population with 0–98% HbF, we observed two differences: 1) the precision had fallen to 5.6%; 2) OSM3 gave values over 100% in five cases). These differences may be due to the fact that the matrix of absorption coefficients used in the OSM3 for neonatal blood was adapted to a limited range of HbF, *i.e.* 70–90%.

Despite these limitations, our results show that the degree of agreement between the two methods of HbF % measurement is acceptable, and accordingly, that HbF determination by the OSM3 appears to be useful for clinical practice. Because high percentages of HbF can affect the measurement of Sao_2 by co-oxymeter, CORNELISSEN *et al.* [7] introduced correction factors for haemoglobin derivatives in fetal blood. An accurate determination of HbF by a rapid method is, therefore, useful to provide a better estimation of oxygenation in newborn infants in intensive care units, especially when testing the reliability of determination of transcutaneous oxygen saturation by pulse oxymetry [8, 9]. We suggest that the OSM3 will be a useful clinical instrument for this purpose.

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